学校编码：10384　　分类号_____密级_____

学　　号：19220051301772　　UDC_____

厦门大学

硕　士　学　位　论　文

芳樟叶黄酮和多糖的提取分离、结构鉴定及抗氧化活性研究

Extraction, Separation, Identification and Antioxidant activities of Flavonoid and Polysaccharide from *Cinnamomum Camphora* Leaves

王　先

指导教师姓名：　李　清　彪　　教　授

王　远　鹏　　助理教授

专　业　名　称：　生　物　化　工

论文提交日期：　２００８　年　**9**　月

论文答辩日期：　２００８　年　**9**　月

学位授予日期：　２００８　年　　月

答辩委员会主席：_____

评　　阅　　人：_____

2008 年 8 月

厦门大学学位论文原创性声明

本人呈交的学位论文是本人在导师指导下，独立完成的研究成果。本人在论文写作中参考其他个人或集体已经发表的研究成果，均在文中以适当方式明确标明，并符合法律规范和《厦门大学研究生学术活动规范（试行）》。

另外，该学位论文为（　　）课题（组）的研究成果，获得（　　）课题（组）经费或实验室的资助，在（　　）实验室完成。（请在以上括号内填写课题或课题组负责人或实验室名称，未有此项声明内容的，可以不作特别声明。）

声明人（签名）：

年　　月　　日

厦门大学学位论文著作权使用声明

本人同意厦门大学根据《中华人民共和国学位条例暂行实施办法》等规定保留和使用此学位论文，并向主管部门或其指定机构送交学位论文（包括纸质版和电子版），允许学位论文进入厦门大学图书馆及其数据库被查阅、借阅。本人同意厦门大学将学位论文加入全国博士、硕士学位论文共建单位数据库进行检索，将学位论文的标题和摘要汇编出版，采用影印、缩印或者其它方式合理复制学位论文。

本学位论文属于：

（　　）1.经厦门大学保密委员会审查核定的保密学位论文，于　　年　　月　　日解密，解密后适用上述授权。

（　　）2.不保密，适用上述授权。

声明人（签名）：

年　　月　　日

摘要

我国是世界上生产芳樟油最多的国家，其产量占世界的80%。樟树（*Cinnamomum camphora*）是我国生产天然香料芳香油的主要树种，经济价值高。近年来，通过组培、扦插等方法培育出纯种芳樟，大大提高樟树资源的经济价值。但在提取精油过程中产生数量巨大的芳樟枝叶等废弃物，由于对芳樟废弃物的资源化研究力度不够，忽视了许多有药用价值的化学成分如黄酮、多糖等化合物的开发利用，造成芳樟资源浪费严重，有必要改进和发展芳樟中有效成分的提取纯化工艺以及寻找其中有生物活性的化学成分，为芳樟资源的综合利用提供理论与实践依据，发挥其最好的社会效益和经济效益。

通过对芳樟树不同部位的测定发现，不同部位中总黄酮含量和FRAP值均有明显差异，其中芳樟叶的总黄酮含量和FRAP值最高。不同部位中总黄酮含量与FRAP值呈正相关性。高效液相色谱法（HPLC）测定结果表明芳樟叶中黄酮苷元主要含有槲皮素、山奈素和异鼠李素，其含量分别为：0.39%、0.14%和0.063%，在芳樟叶中大部分以黄酮糖苷的形式存在。

通过单因素实验和正交试验优化了芳樟叶黄酮的微波辅助萃取工艺条件：乙醇浓度60%（v/v），料液比1：12（g/mL），微波功率320 W，间歇辐射两次，每次1 min，芳樟叶黄酮提取得率达2.97%，比乙醇热回流提取提高了6.83%，并且提取时间缩短了近99%；为了进一步纯化芳樟叶黄酮，通过测定10种大孔树脂对芳樟叶黄酮的静态吸附量和解吸率，确定了芳樟叶黄酮纯化工艺的吸附剂DA201树脂，并优化了DA201树脂对芳樟叶黄酮的动态吸附和解吸工艺条件：上柱液黄酮浓度为13.20 mg/mL，树脂柱径高比为1：10，吸附流速为3 BV/h；洗脱溶剂为5 BV 50%的乙醇水溶液，洗脱流速为3 BV/h。芳樟叶粗提液经装填有大孔树脂DA201的固定床纯化后黄酮含量由38.15%提高到91.18%，纯化倍数2.39倍。

采用HPLC法测定芳樟叶精制黄酮中芦丁、槲皮素、山奈酚和异鼠李素的含量，分别为123.33 mg/g、70.00 mg/g、28.63 mg/g和20.81 mg/g。利用高效液相色谱－二极管阵列检测器－电喷雾质谱（HPLC-DAD-ESI-MS）联用技术对芳樟叶精制黄酮的主要组分进行了定性分析，初步鉴定出芦丁、槲皮素-3-O-β-D-半乳糖苷、山奈酚-3-O-β-D-芸香糖苷、异鼠李素-3-O-β-D-芸香糖苷、槲皮素-3-O-β-D-鼠李糖苷、山奈酚-3-O-β-D-鼠李糖苷6种黄酮糖苷。采用Sephadex LH-20

凝胶柱从精制黄酮中分离得到芦丁和山奈酚-3-O-β-D-芸香糖苷。

进一步采用沸水提取、乙醇沉淀的方法，从芳樟叶中提取得到水溶性粗多糖CLPS。经DEAE52-纤维素柱和Sephacryl-S400凝胶柱分离得到一种酸性多糖组分CLPS-A2，采用高效凝胶渗透色谱法测定其为均一性多糖，平均分子量为244 KDa。紫外可见吸收光谱和红外光谱的分析结果表明CLPS-A2为具有乙酰氨基结构的β型吡喃多糖。CLPS-A2水解产物的分析结果显示，CLPS-A2是一种主要由鼠李糖、木糖、阿拉伯糖、葡萄糖、半乳糖组成的酸性杂多糖。

利用 FRAP 法和 DPPH 法测定了芳樟叶各提取物的体外抗氧化活性。其中，芳樟叶总黄酮苷元的抗氧化活性最强，其次是芳樟叶精制黄酮，芳樟叶多糖的抗氧化活性最弱。结合对黄酮结构的分析可知，芳樟叶的抗氧化活性与其所含的黄酮醇苷有关。

关键词：芳樟叶；黄酮；多糖；提取；分离；结构鉴定；抗氧化活性

ABSTRACT

Cinnamomum camphora is an important plant that has been used to produce natural flavor and aromatic oils in China for a long time. In recent years, it was reported that purebred *Cinnamomum camphora* could be successfully cultivated by tissue culture, which significantly enhanced the value of *Cinnamomum camphora*. However, considerable components of pharmacological merit in *Cinnamomum camphora*, e.g. flavonoid, polysaccharide, received little attention. To utilize *Cinnamomum camphora* trees more reasonably and economically, the techniques for extracting pharmacological components from *Cinnamomum camphora* trees should be improved and exploited.

A large variation of total flavonoid contents and FRAP values in different parts of *Cinnamomum camphora* trees were observed. The *Cinnamomum camphora* leaves showed to be of the highest total flavonoid content and FRAP value. A positive linear correlation between FRAP values and total flavonoid contents were established. After the acid-catalyzed hydrolysis of the leaves quercetin(0.39%), kaempferol(0.14%) and isorhmnetin(0.063%) were detected and founded to exist mainly in the form of flavonoid glucosides.

By a microwave-assisted extraction technology flavonoid was extracted from the residue of *Cinnamomum camphora* leaves after essential oil extraction. Macroporous resin adsorption was used for further purification. With single factor and orthogonal designed experiments the optimal extraction conditions were determined as follows: 60% ethanol aqueous solution, ratio of solid to liquid 1:12(g/mL), microwave power 320 W and treated for 2 min,. Under such conditions, the yield of the flavonoid reached 2.97%, which was 6.82% higher than that obtained using heat ethanol reflux extraction, and the extraction operation duration of the technology was significantly reduced to 1% of that for the control process. Ten kinds of macroporous resin were then examined for further purification of *Cinnamomum camphora* flavonoid. DA201 resin was showed to possess the best absorption and desorption behavior. The optimum adsorption and desorption conditions were sample solution flavonoid

concentration 13.20 mg/mL, diameter to heighth 1:10, absorption rate 3BV/h; The optimum desorption conditions were volume of 50% ethanol 150 mL (approximately 5 BV) as eluting solvent, eluting rate 3BV/h. The content of flavonoid could be improved to 91.18% from 38.15% after an absorption-desorption operation applied upon a DA201 resin packed column.

By HPLC, rutin(123.33 mg/g), quercetin(70.00 mg/g), kaempferol(28.63 mg/g), isorhmnetin(20.81 mg/g) were identfied and determined in the purified flavonoid obtained above. By HPLC-DAD-ESI-MS, six flavonoid glycosides, rutin, quercetin-3-O-β-D-galacoside, kaempferol-3-O-β-D-rutinoside, isorhmnetin-3-O-β-D-rutinoside, quercetin-3-O-rhamnoside and kaempferol-3-O-β-D-rhamnoside were primarily identified in the purified flavonoid. Rutin and kaempferol-3-O-β-D-rutinoside were separated from the purified flavonoid by sephadex LH-20 column chromatography.

The crude water-soluble polysaccharide (CLPS) was obtained from *Cinnamomum camphora* leaves by boiled water extraction and ethanol precipitation. DEAE52-cellulose column and Sephacryl-S400 gel filtration column were used to further separate an acid polysaccharide (CLPS-A2) from the crude polysaccharide. The molecular weight of CLPS-A2 was determined to be 244 kDa. UV-VIS spectrum and FTIR analysis showed that CLPS-A2 was β-heterosaccharides with pyran and acetyl amino group. Analysis of the hydrolysis products of CLPS-A2 showed that CLPS-A2 was a heterosaccharide composed of rhamnose, arabinose, xylose, glucose and galactose.

The antioxidant activities of various extracts from *Cinnamomum camphora* leaves were evaluted by FRAP assay and DPPH assay in vitro. The result showed that the antioxidant activities of various extracts ranged as the following order: total flavonoid-aglycone>purified flaovnoid>polysaccharide. It was inferred that the potent antioxidant activities of *Cinnamomum camphora* leaves were mainly attributed to flavonol glycosides.

Key words: *Cinnamomum camphora*; flavonoid; polysaccharide; extraction; separation; identification; antioxidant activities

目录

Contents

第一章 文献综述

1.1 樟树简介

樟树（*Cinnamomum camphora*），别名：香樟、樟木、乌樟、油樟、香樟、芳樟。樟树是樟科（*Lauraceae*）樟属（*Cinnamomum*）植物，系常绿乔木，单株高度可达 30 米，树冠广卵形；树皮幼时绿色，平滑，老时渐变为黄褐色或灰褐色纵裂，冬芽卵圆形。叶互生，革质薄，呈卵状椭圆形，长 6～12 厘米，宽 3～6 厘米，顶端短尖或近尾尖，基部圆形，背面呈灰绿色，两面无毛，有离基三出脉，近叶基的第一对或第二队侧脉长而显著，脉腋有明显的腺体。圆锥花序腋生于新枝，长 5～7.5 cm；花小，淡黄绿色；花被 6 片，椭圆形，长约 2 毫米，内面密生短柔毛；能育 9 个雄蕊，花药 4 室；第三轮雄垒花药外向瓣裂；子房球形，无毛。核果球形，直径 6-8 mm，熟时紫黑色；果托杯状。花期 5 月，果实 9-11 月成熟。

樟树是我国生产天然香料芳香油的主要树种，同时也是珍贵用材树种及园林绿化的优良树种。樟树是热带和亚热带常绿阔叶林的代表树种，被誉为江南宝树。其分布区广，分布区域在北纬 10º～34º，东经 88º～120º，主产于我国海南、台湾、福建、江西、广东、广西、湖北、湖南、四川、重庆、云南、贵州、浙江等省区，以福建、台湾为最多。樟树的栽培利用早在东周春秋时代就有记载，至今已有三千多年的栽培历史。樟树用途广，经济价值高，可谓全身都是宝，其主副产品广泛应用于建筑、工艺、化工、军工、医药等方面。其木材致密美观，具有芳香味，抗虫蛀，可供建筑、造船、家具、乐器、手工艺品之用；樟树的茎叶均可提制樟脑、樟油，油中含有桉叶素、黄樟素、芳樟醇、松油醇、柠檬醛等多种重要成分，是国防、医药、化工香料等工业的重要原料。芳樟油减压精馏所得的第二馏分芳油，其主要成分是芳樟醇，为许多高贵香料的主要原料，既可直接用于调香，又可制造柠檬醛合成紫罗兰香酮，还可制天竺葵醇和乙酸芳樟酯等。樟树树姿秀丽、四季常青、枝叶茂密，具有很强的吸尘能力的抗煤烟能力；且系深根性树种，根系特别强大，主根尤为发达，有强的抗风能力；且能吸湿耐水、防风固沙、保护堤岸，近年来被广泛选作南方城镇及“四旁”绿化树种和环保优良树种。

樟树是樟科植物中枝叶含芳樟醇最高的一种。芳樟醇原料及其派生衍生物的